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The Haptoglobin 1 Allele Correlates With White Matter Hyperintensities in Middle-Aged Adults With Type 1 Diabetes

COMPLICATIONS

Although the haptoglobin (Hp) 1-1 genotype is associated with a lower coronary artery disease (CAD) risk in diabetes, we recently reported an increased stroke incidence in type 1 diabetes with Hp 1-1. We, thus, evaluated differences in earlier brain vascular abnormality markers by Hp using neuroimaging. Neuroimaging was completed in 94 participants of the Pittsburgh Epidemiology of Diabetes Complications study with Hp genotyping available (mean age, 49; duration, 41 years). White matter hyperintensities (WMH) volume, lacunar infarcts, and gray matter atrophy were quantified. Sixteen percent were homozygous for Hp 1 and 43% for Hp 2. A significant trend toward increased WMH was observed with greater duration and the number of Hp 1 alleles. Associations were strongest for the interhemispheric connecting fibers of the corpus callosum. Allowing for duration, sex, waist-to-hip ratio, HbA$_{1c}$, systolic blood pressure, and lipids in models with backward elimination, results were similar. No significant differences by Hp were noted for atrophy or lacunar infarcts. Consistent with its direct association with stroke, the Hp 1-1 genotype is associated with higher WMH in this population. Further, including mechanistic, studies on the role of the Hp genotype in cerebrovascular disease and the implications for worsening cognitive function are needed.

White matter hyperintensities (WMH), a cerebrovascular abnormality identifiable via brain MRI, are typical features of the aging brain, are neither benign nor reversible, and predict greater risk of dementia, disability, stroke, and mortality (1). Whether WMH respond to treatment is unknown, although their progression could potentially be delayed (2).

A recent general population study suggested a lower prevalence of the haptoglobin (Hp) 2 allele in stroke (3). Another study, among hypertensive patients without symptomatic vascular disease, noted greater WMH with Hp 1 (4). These findings were surprising given multiple prospective studies in diabetes pointing to increased cardiovascular disease (CVD) risk with Hp 2 allele homozygosity (5–9).

Hp is an acute-phase protein whose major function is binding to free hemoglobin, thus acting as an antioxidant (10). In humans, two classes of alleles exist at the Hp locus (16q22), Hp 1 and Hp 2 (resulting from duplication of exons 3 and 4 [10]), yielding three genotypes (Hp 1-1, 2-1, and 2-2). These allelic protein products differ in structure and function, with Hp 2-2 being a less efficient antioxidant (10,11).

In the Pittsburgh Epidemiology of Diabetes Complications (EDC) study of type 1 diabetes, we observed an increasing coronary artery disease (CAD) incidence with the number of Hp 2 alleles (12) and, interestingly, an inverse association with stroke (13). To further comprehend these discrepant findings, we sought to determine whether the Hp genotype was associated with markers of cerebrovascular abnormalities, including WMH and lacunar infarcts.

RESEARCH DESIGN AND METHODS

The EDC is a representative (14), prospective investigation of a historical, incident childhood-onset type 1

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diabetes cohort, diagnosed or seen within 1 year of diagnosis (1950–1980) at Children's Hospital of Pittsburgh (15). After the initial examination (1986–1988), biennial surveys and examinations followed for 24 years. During the 24-year follow-up, participants were invited to a neuroimaging substudy. Of 149 (56.7%) accepting the invitation, 112 were eligible and 95 had usable WMH data; 94 also had Hp genotyping. All participants provided informed consent. The University of Pittsburgh Institutional Review Board approved all study protocols.

Participants underwent brain MRI in a 3T scanner. Two sequences were captured (T1-magnetization prepared rapid acquisition gradient echo and T2-weighted fluid attenuated inversion recovery [FLAIR]). An automated labeling pathway WMH segmentation method was used to quantify volumes and localization of focal WMH (16). Total brain volume constituted the sum of gray and white matter volumes and cerebrospinal fluid obtained using an automated labeling pathway. Tracts examined included the corpus callosum and long connecting tracts vulnerable to cardiovascular risk factors, comprising the superior and inferior longitudinal fasciculus, anterior thalamic radiation, corticospinal tracts, and cingular bundle.

Chronic lacunar-type infarcts were distinguished from WMH and prominent perivascular spaces by careful review. Infarcts were defined as T2 hyperintense/T1 hypointense lesions with irregular borders and central parenchymal loss, which was suppressed on T2/FLAIR sequences. T2 WMH, commonly attributed to chronic small vessel ischemic disease, demonstrated no parenchymal loss and did not suppress centrally on the T2/FLAIR sequences. Prominent perivascular spaces, which can demonstrate central T1 hypointensity and suppression on T2/FLAIR sequences, were distinguished from infarcts by smooth borders, a linear orientation, and the absence of adjacent T2 hyperintensity. Ratings were done by a board-certified neuroradiologist (J.M.M.).

Smoking status was self-reported. Blood pressure was measured with a random zero sphygmomanometer after a 5-minute rest (17), and hypertension was defined as ≥140/90 mmHg or antihypertensive medication use. Glycosylated hemoglobin (HbA$_{1c}$; DCA 2000 analyzer, Bayer HealthCare, Tarrytown, NY), nonfasting total and HDL cholesterol (HDLc; Cholestech LDX Analyzer, Cholestech Corporation, Hayward, CA), and the urinary albumin-to-creatinine ratio (DCA Vantage Analyzer, Siemens Healthcare Diagnostics) were also measured. Non-HDLc was calculated as total cholesterol minus HDLc. Hp was genotyped by an amplification method (18). When DNA was unavailable, Hp phenotype was assessed (19).

CAD was determined by study-physician diagnosed angina, ischemic electrocardiogram changes (Minnesota codes 1.3, 4.1–4.3, 5.1–5.3, 7.1), revascularization, confirmed myocardial infarction (Minnesota codes 1.1/1.2 or hospital records), or CAD death. Stroke was defined as an acute-onset 24-h neurological deficit, without other evident cause, confirmed by hospital/autopsy records.

Statistical Analysis

Univariate differences by Hp were determined with descriptive statistics. The independence of the Hp-WMH association was evaluated with general linear models, using Bonferroni adjustment for multiple comparisons by Hp and α = 0.008 to account for assessing multiple tracts.

RESULTS

Hp 1 homozygosity was 16% and Hp 2 was 43%. Participant characteristics were similar by Hp, although diabetes duration was longer, non-HDLc was lower, and, among women, HDLc was higher with Hp 1-1 (Table 1). CAD incidence increased from Hp 1-1 to 2-2. Only one participant (Hp 2-1) experienced a clinical stroke; three (one of each Hp type) had a lacunar infarct. All but one (Hp 2-2) had measurable total brain WMH, which increased in extent with duration (Fig. 1A). The extent of WMH increased with diabetes duration to a greater degree in Hp 1-1 (r = 0.49, P = 0.06) than in Hp 2-1 (r = 0.25, P = 0.12) or 2-2 participants (r = 0.15, P = 0.35; Fig. 1B). A direct association between total WMH and Hp 1 allele number was observed (P for trend = 0.02, Table 1), resulting mainly in the interhemispheric connecting fibers of the corpus callosum (P for trend = 0.004). No differences by Hp were seen in other tracks or gray matter atrophy. Figure 2 shows the MRI scan results of an Hp 1-1 and an Hp 2-2 participant of similar age and diabetes duration. Hyperintensities in subcortical and periventricular areas with substantial ventricular enlargement are evident in the Hp 1-1 participant's scan. Hyperintensities are restricted to the anterior periventricular areas of the Hp 2-2 participant, lacking ventricular enlargement.

In regression models, diabetes duration explained 14% of the total brain WMH variation, whereas Hp contributed a further (nonsignificant) 2.5% (Table 2). For total corpus callosum WMH, duration explained 19.5% of the variance, whereas Hp contributed a further 4% (P = 0.03). Allowing for duration, sex, HbA$_{1c}$ and lipids, results were similar despite the reduction in sample size (n = 88; $P_{\text{total brain}}$ = 0.2887, $P_{\text{total corpus callosum}}$ = 0.0862).

Because diabetes duration appeared to drive most of the WMH variation and most of the participants with longer duration carried the Hp 1 allele (Fig. 1B), we sought to evaluate whether duration modified the Hp-WMH relationship. No interaction was observed for total brain WMH (P = 0.35), although a borderline significant effect modification was observed for total corpus callosum WMH (P = 0.07).

DISCUSSION

In this study of middle-aged adults with type 1 diabetes, the Hp 1 allele was associated with greater WMH localized in the interhemispheric connecting fibers of the corpus callosum. This association appeared to be largely driven by diabetes duration, which strongly correlated with WMH and the presence of at least one Hp 1 allele. However, although adjusting for duration rendered the

Table 1—Participant characteristics by Hp genotype

	Hp 1-1 (*n* = 15)	Hp 2-1 (*n* = 39)	Hp 2-2 (*n* = 40)	*P* value
Age (years)	52.7 (8.8)	49.7 (6.8)	48.0 (6.0)	
Age at onset (years)	7.5 (4.6)	9.1 (4.1)	7.6 (4.1)	
Duration (years)	45.2 (7.9)	40.6 (6.4)*	40.4 (5.3)*	
Females (%, *n*)	53.3 (8)	56.4 (22)	40.0 (16)	0.32
BMI (kg/m^2)	25.5 (3.7)	26.4 (4.7)	28.4 (4.7)	
Waist-to-hip ratio				
Men	0.92 (0.05)	0.92 (0.07)	0.97 (0.07)†	
Women	0.78 (0.10)	0.85 (0.07)	0.85 (0.07)	
Ever smoker (%, *n*)	40.0 (6)	21.6 (8)	21.0 (8)	0.33‡
HbA$_{1c}$ (%)	7.4 (1.4)	8.1 (1.6)	7.4 (1.2)	
Blood pressure (mmHg)				
Systolic	122.8 (15.7)	114.8 (14.5)	120.7 (14.4)	
Diastolic	63.3 (12.7)	65.1 (7.5)	65.8 (9.8)	
Pulse (bpm)	67.5 (14.0)	68.6 (11.7)	68.5 (12.7)	
Hypertension (%, *n*)	20.0 (3)	18.4 (7)	21.6 (8)	0.94‡
HDLc (mg/dL)				
Men	41.6 (6.9)	50.2 (14.3)	48.0 (14.7)	
Women	88.0 (16.5)	70.6 (17.2)	57.3 (19.7)*	
Non-HDLc (mg/dL)	96.1 (26.8)	96.4 (26.2)	116.7 (36.9)†	
Albumin-to-creatinine ratio (mg/g)	20.2 (9.6, 61.1)	12.7 (7.5, 35.6)	11.2 (5.4, 28.6)	0.16
CAD (%, *n*)	0.0 (0)	12.8 (5)	25.0 (10)	0.06‡
Stroke (%, *n*)	0.0 (0)	2.2 (1)	0.0 (0)	1.00‡
Lacunar infarcts (%, *n*)	6.7 (1)	2.2 (1)	2.4 (1)	0.53‡
White matter areas ($\times 10^4$)				
Total brain WMH§	12.527 (7.372, 24.582)	8.345 (6.078, 15.164)	8.617 (5.427, 12.687)	0.02**
Total corpus callosum WMH§	3.916 (2.283, 8.449)	2.685 (1.219, 6.122)	2.027 (1.069, 2.977)	0.004**

Continuous data are shown as means (SD) or median (interquartile range) and categoric data as indicated. *Significantly different from Hp 1-1 after Bonferroni adjustment for multiple comparisons. †Significantly different from Hp 2-1 after Bonferroni adjustment for multiple comparisons. ‡Fisher exact test *P* value. §Logarithmically transformed variables were used. **P* value for trend.

Hp–total brain WMH association nonsignificant, it did not eliminate the Hp–total corpus callosum WMH association.

During the past decade, Hp has been emerging as a major risk factor for the development of diabetic CVD, with most studies having focused on type 2 diabetes. Indeed, five longitudinal studies showed significantly increased cardiovascular risk with Hp 2-2, although a similar association has generally not been observed in nondiabetic populations (5–9). Moreover, despite numerous trial failures to demonstrate cardiovascular benefit with antioxidant supplementation, three trials provided evidence that the protective effect of vitamin E therapy against CVD is restricted to the subgroup with type 2 diabetes and Hp 2-2 (20).

To date, the EDC has been the only study to have evaluated the association between the Hp genotype and CAD incidence in type 1 diabetes, demonstrating increasing risk with the number of Hp 2 alleles (12). Interestingly, although a similar association has generally not been observed in the general population, a greater frequency of the Hp 1 allele in a first symptomatic lacunar stroke was reported in a population with high hypertension prevalence (82%), only 11% of whom had diabetes (3). Furthermore, among hypertensive patients, Hp 1-1 was suggested to be cross-sectionally associated with larger deep white matter lesions (4). Although the nature of these general population study designs allows questions regarding survival bias having affected findings, prospective analyses from our EDC study also suggest a greater incidence of stroke with Hp 1-1 in type 1 diabetes (13), although the number of incident cases was small. Herein, we further report that the extent of WMH in the brain appears linked to the presence of the Hp 1 allele, which correlated with WMH localized in the interhemispheric connecting fibers of the corpus callosum. Thus, although the associations presented here are cross-sectional and we cannot rule out the possibility that residual confounding, competing risk, and/or survival bias have distorted the "true" association between the Hp genotype and extent of WMH in the brain, the prospective relationship between Hp and stroke in EDC demands that we do not readily dismiss these cross-sectional results.

Given the strength and abundance of evidence demonstrating a protective effect of the Hp 1 allele for
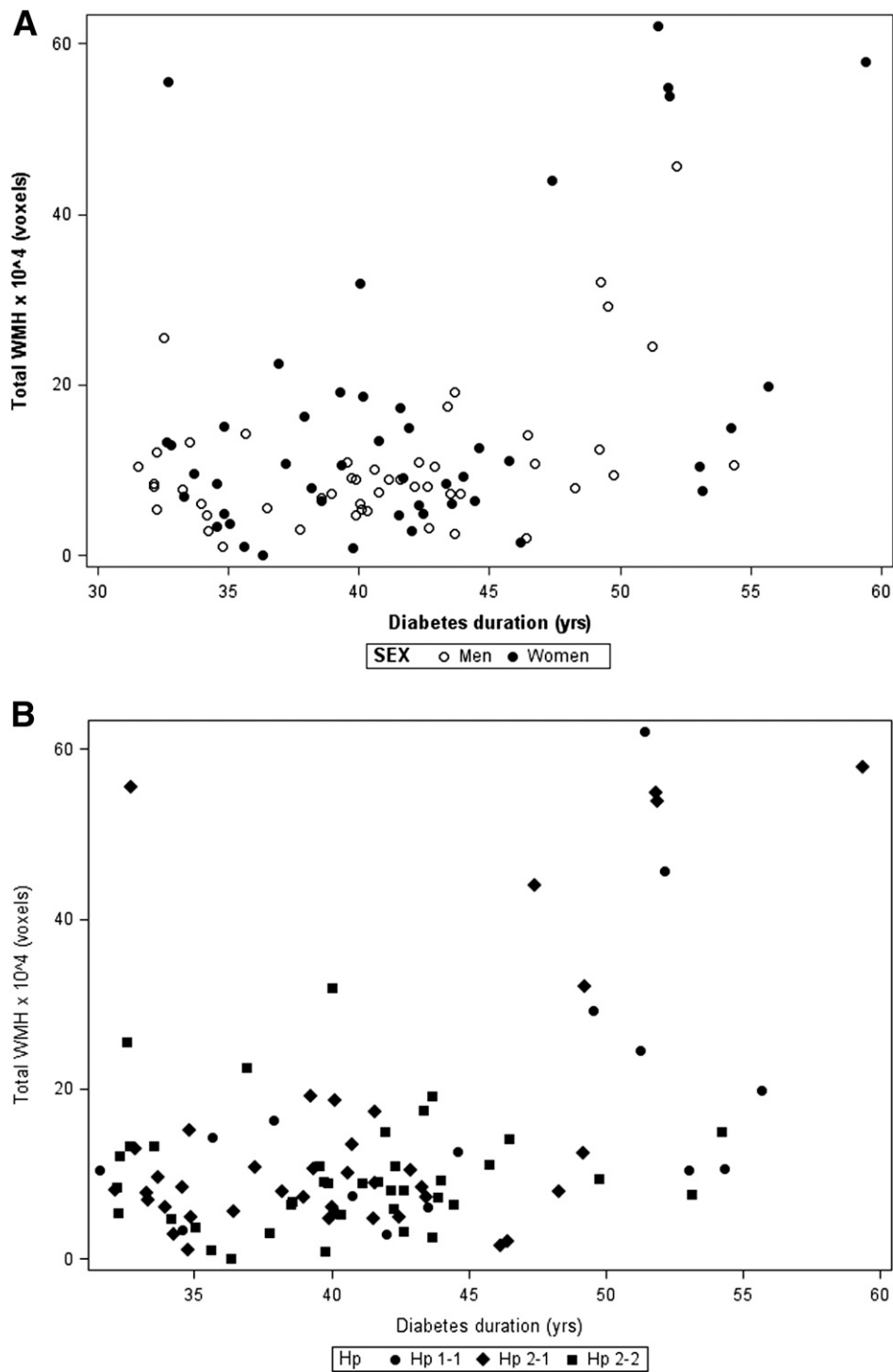
Figure 1—*A*: Total WMH in men and women with type 1 diabetes. *B*: Total WMH by Hp genotype and duration of type 1 diabetes.

vascular diabetes complication incidence, however, it is difficult to comprehend the suggested increased risk conferred by the same allele in terms of cerebrovascular disease. If true, these apparently contradictory findings likely relate to existing functional differences between the two Hp allelic protein products as well as hyperglycemia, a distinct feature of diabetes. Most hypotheses and

investigations of Hp thus far have focused on associations relating to its major function: the binding of free hemoglobin and its removal from circulation, where the Hp 1 allele has an advantage. Thus, the protection conferred by the Hp 1-1 genotype against CAD development is thought to be mediated by the greater antioxidative and anti-inflammatory capacity of the Hp 1 allele
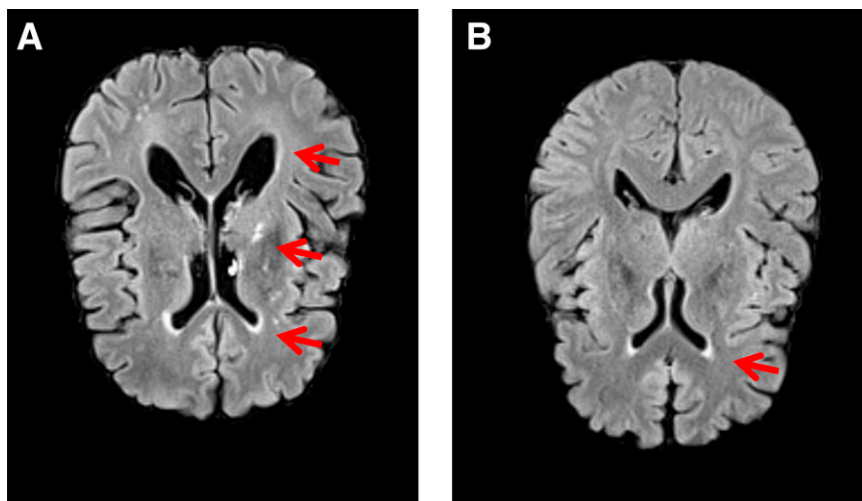
Figure 2—Brain MRI scan results of one individual with Hp 1-1 and another with Hp 2-2. *A*: FLAIR image of a 58.6-year-old individual with type 1 diabetes for 50 years and the Hp 1-1 genotype shows hyperintensities in subcortical areas and periventricular areas, with substantial ventricular enlargement (arrows). *B*: FLAIR image of a 55.6-year-old individual with type 1 diabetes for 53 years and the Hp 2-2 genotype shows hyperintensities only restricted to the anterior periventricular areas (arrow) but not in the parenchyma and no signs of ventricular enlargement.

(11). These Hp properties are thought to be of greatest importance in the presence of impaired glucose tolerance for a number of reasons, including the reduced Hp efficiency to prevent oxidation from HbA_{1c} (11).

The importance of Hp to normal physiology, however, expands beyond its role as the scavenger of free hemoglobin. Indeed, Hp represents a potent angiogenic factor, essential for the proliferation and differentiation of endothelial cells in neovascular development (21). Moreover, although Hp is primarily produced by liver hepatocytes (10), Hp is also expressed in the brain, and thus, disruption of the blood-brain barrier is not the only pathway of Hp entry in the brain (22). Interestingly, compared with the other two genotypes, Hp 2-2 has been associated with enhanced angiogenic activity (21). A recent publication (23) shed light on the potential mechanism by which this Hp property may relate to the observed associations with WMH and stroke. It is well established that endothelial dysfunction has a major role in the development and progression of diabetes complications, potentially also including cerebral small vessel disease (24), which underlies white matter lesion and lacunar infarct formation. Investigators suggested that the formation of endothelial cell clusters, which may mitigate cerebral small vessel disease, appeared attenuated in lacunar stroke patients with Hp 1-1 versus Hp 2-2 (23). In addition, cluster numbers were lower when Hp 1-1 was added to endothelial progenitor cell cultures compared with numbers produced by adding other Hp types, suggesting that Hp 1-1 may provide inadequate protection against small vessel disease initiated subsequent to endothelial damage, thus perhaps promoting greater WMH and stroke. Whether this increased angiogenesis after a stroke event continues to be beneficial via formation of functional new blood vessels after the insult is unclear (25), however, given prior evidence that neovascular growth may also lead to pathologic conditions (e.g., diabetic retinopathy) (26).

Table 2—General linear regression models for total brain and total corpus callosum WMH with the number of Hp 1 alleles (*n* = 94)

Outcome	Independent variable	β (SE)	*P* value	Model R^2
Total brain WMH*	Model 1			0.1401
	Diabetes duration	0.05 (0.01)	0.0002	
	Model 2			0.1648
	Diabetes duration	0.04 (0.01)	0.0008	
	Number of Hp 1 alleles	0.17 (0.11)	0.1054	
Total corpus callosum WMH*	Model 1			0.1954
	Diabetes duration	0.05 (0.01)	<0.0001	
	Model 2			0.2346
	Diabetes duration	0.04 (0.01)	<0.0001	
	Number of Hp 1 alleles	0.20 (0.09)	0.0281	

*Logarithmically transformed before statistical testing.

Undoubtedly, further studies on the role of the Hp genotype in cerebrovascular disease are needed to elucidate the pathophysiology and clinical implications of these associations and possible therapeutic interventions. Moreover, our own findings of diverse effects of Hp on CAD and stroke require validation. It is intriguing, however, to speculate that the failure to demonstrate protection against CVD with vitamin E in the absence of the Hp 2-2 genotype in diabetes may be at least partly explained by an increased stroke risk associated with the Hp 1 allele (20) because vitamin E may increase the risk of bleeding. Lastly, it is becoming evident that the Hp genotype asks us to tell a more intricate story than perhaps originally anticipated, one that will certainly make the search for answers exhilarating.

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